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No sexual differences in embryonic period in jackdaws *Corvus monedula* and black-headed gulls *Larus ridibundus*

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Offspring survival probability usually decreases with hatching order, especially in species with brood reduction. Brood reduction in combination with a sex difference in embryonic period (the time between laying and hatching of an egg) can potentially have a profound effect on sex allocation, with higher investment in chicks of the early hatching sex because they are more likely to survive to fledge. Two recent studies reported sex differences in the embryonic period, but compared embryonic period between, rather than within, clutches, which does not control for possible environmental effects on both clutch sex ratio and embryonic period. We compared the embryonic period of sons and daughters within clutches in jackdaws *Corvus monedula* and black-headed gulls *Larus ridibundus*, two species with frequent brood reduction, and found no sexual difference in embryonic period. This suggests that sex allocation is not affected by sex differences in embryonic period in these species, but more studies are required to verify whether this is a general pattern.

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Sex allocation, the division of parental investment between sons and daughters (Fisher 1930, Frank 1990), is an important aspect of life history theory that is receiving increasing attention since the introduction of molecular techniques to sex birds (Griffiths and Tiwari 1993, Fridolfsson and Ellegren 1999). Since offspring survival probability usually decreases with hatching order in species with brood-reduction (O'Connor 1978, Mock and Parker 1997), variation in embryonic period within clutches can have a large effect on the division of parental investment between chicks (Gibbons 1987). Consequently, a sexual difference in embryonic period can potentially have a strong effect on sex allocation. A sexual difference in embryonic period could arise either through an inherent difference between the sexes in the rate of development (Kraak and de Looze 1993), or when females modify egg characteristics that affect embryonic period depending on the sex of the embryo (e.g. androgen concentration; Eising et al. 2001). Adjust-

ment of sex allocation via the embryonic period could act in concert with previously described mechanisms, such as biases in the primary sex ratio (Sheldon 1998, Pike 2005) or systematic biases of the sex ratio with respect to the laying order (Dijkstra et al. 1990), which affects hatching order and thereby competitive ability and post hatching survival of the sexes.

Despite the potential effects on sex allocation, sex differences in embryonic period have to our knowledge been little studied. However, two recent papers reported that in the eurasian kestrel *Falco tinnunculus* (Blanco et al. 2003) and the black guillemot *Cephus grylle* (Cook and Monaghan 2004), the embryonic period differed between sons and daughters. The observed differences were 2.0 h (females faster; See Table 1 in Blanco et al. 2003) and approximately 1 day (males faster), respectively. In addition, two studies of domestic fowl *Gallus gallus domesticus* compared embryonic period between the sexes, with one study reporting a 3 h sex difference in

Table 1. Embryonic period in relation to sex, laying date (lay date), year, laying order (lay order) and egg size. Data were analyzed using mixed models. Final model includes all parameters in the column 'included terms' (and a constant). Changes in deviance (Δ dev) and degrees of freedom (df) indicate the changes when parameters are dropped from the final model one at the time (or added to the final model for rejected terms). Sex (0 for females and 1 for males) and egg volume were tested in models containing clutch sex ratio and average egg volume per clutch respectively.

Species	Included terms	Rejected terms	Estimate	SE	Δ dev	df	P
Jackdaw (Incubator) (N = 43 broods)	Lay order				236.621	4	<0.001
	Lay date ^(a)		−0.046	0.117	0.153	1	0.696
	Lay order \times lay date				10.448	4	0.036
	Clutch size		−0.796	0.125	40.555	1	<0.001
	Year ^(b)		−0.400	0.102	15.365	1	0.004
		Sex	0.132	0.104	1.605	1	0.205
		Clutch sex ratio	0.037	0.310	0.014	1	0.906
		Sex \times lay order			4.391	4	0.356
		Egg volume	0.111	0.090	1.521	1	0.217
		Mean egg volume	−0.197	0.133	2.194	1	0.139
Black-headed gull (Incubator) (N = 46 broods)	Lay order				132.914	2	<0.001
	Lay date		−0.300	0.095	9.486	1	0.002
	Lay order \times lay date				9.109	2	0.011
		Sex	−0.043	0.089	0.236	1	0.627
		Clutch sex ratio	−0.086	0.296	0.084	1	0.772
		Sex \times lay order			1.940	2	0.379
		Egg weight	0.012	0.037	0.112	1	0.738
		Mean egg weight	0.020	0.028	0.509	1	0.476
Black-headed gull (Field) (N = 105 broods)	Lay order				212.442	2	<0.001
	Lay date		−0.088	0.092	0.914	1	0.339
	Lay order \times lay date				11.870	2	0.003
	Egg weight		−0.081	0.033	6.104	1	0.013
	Mean egg weight		0.128	0.042	8.940	1	0.003
		Sex	0.087	0.095	0.847	1	0.357
		Clutch sex ratio	0.129	0.243	0.282	1	0.595
		Sex \times lay order			2.188	2	0.335

^(a)Calculated for lay order 1.

^(b)Calculated for 2000.

embryonic period (females faster; Burke 1992), while another study found a nonsignificant trend in the opposite direction (Dunnington et al. 1993). However, comparisons between the sexes in these studies were a mixture of between and within clutch/female comparisons, leaving open the possibility that the sex differences observed were in fact due to environmental or genetic factors which simultaneously affected embryonic period and clutch sex ratio. For this reason, and because of our interest in sex allocation, we compared embryonic period between sons and daughters in jackdaws *Corvus monedula* and black-headed gulls *Larus ridibundus*. These species are of interest in this context, because they have frequent brood reduction (as do kestrels and guillemots) and sex dependent mortality has previously been suggested as an important mechanism determining sex allocation (Dijkstra et al. 1998, Arnold and Griffiths 2003). We made within clutch comparisons, to control for confounding factors that vary on the clutch level. Since sex ratio has been reported to vary systematically with laying order in some species (e.g. Arnold and Griffiths 2003), and as incubation intensity often gradually increases in the course of laying (e.g. Haftorn 1981), we also controlled for laying order in our analyses.

Methods

Jackdaws

We studied free-living jackdaws breeding in 1998 and 2000 in a colony at the Zoological Laboratory in Haren (The Netherlands). See Verhulst and Salomons (2004) for details. Nest boxes (N = 36) were checked daily, starting the first week of April, and eggs were individually marked as they were laid. Clutches contained 3 to 6 eggs. Eggs are laid between 6:00 and 9:00 AM (CET), and females started incubating the eggs after laying the third egg. To determine from which egg a chick had hatched we moved clutches to an incubator 1–2 days before the estimated hatching date (temperature 37.7°C, humidity 75%). While in the incubator, clutches were replaced with hard-boiled quail eggs, which were readily accepted by the parents. Length and width of the eggs were measured to the nearest 0.1 mm, and egg volume (V , in cm^3) was estimated using the formula: $V = \pi A^2 L K / 6$, where A is width, L is length and for jackdaws $K = 0.00096$ (Soler 1988). Eggs in the incubator were checked at least every 2 h during the day light period. The embryonic period of each egg was defined as the time between the start of incubation (defined as 24:00 h of the day the third egg was laid) and the moment the

egg hatched (i.e. head was out of the egg). Following Blanco et al. (2003), we used the day before the laying of the penultimate egg as start of incubation to calculate the embryonic period. Since we are primarily interested in within clutch variation, the day on which assume incubation starts has no effect on the results. However, when we calculate embryonic period for each egg as the time elapsed between laying and hatching using the laying date of each individual egg the same results emerge. Hatchlings were weighed and a blood sample (20 µl) for sexing was taken by clipping a toenail before they were returned to the nest.

Black-headed gulls

Black-headed gulls are monogamous colonial breeders that typically lay clutches of three eggs (Cramp and Simmons 1983). In 2000 and 2001 nests in a colony of approximately 2000 breeding pairs distributed over several sub-colonies along the northeast coast of The Netherlands were checked daily for egg laying (see also Müller et al. 2005). Freshly laid eggs were marked referring to the position within the laying order and date of egg laying. Black-headed gulls start to incubate the eggs immediately after the first egg is laid. Embryonic period was studied using eggs hatching in their original nest (in 2000), and in incubators (in 2001). At the day of clutch completion eggs were weighed to the nearest 0.1 g. In 2001 the eggs were moved to an incubator (37.5°C, 60% humidity) four days before the estimated hatching date. Around hatching all clutches in the field were checked daily, and in the incubator three to four times per day. In the field, partly hatched chicks were marked on the bill. Hatchlings were marked and bled (20 µl) for a DNA-sample immediately when found. Incubation period was defined as the time between the laying of the first egg and the moment a chick was found hatched. We used only clutches with three eggs, where laying position was known with certainty, and where all chicks could be accurately assigned to their eggs.

Molecular sex identification

Sexes were determined by PCR analysis (for details see Griffiths et al. 1998). The reliability of this method was tested with adult birds of known sex, yielding a 100% correct match in both species (see also Müller et al. 2005).

Statistical analysis

We analyzed our data using mixed models, with normal error distribution, to avoid pseudo-replication. For the analysis of the gull data we included clutch as random effect. For the jackdaw the situation is slightly more

complex, since we collected data of some pairs in both years, and these are not independent. We therefore included both clutch and breeding pair as random effects. Statistical significance of variables was assessed from the increase in deviance (Δdev) when the variable was removed from the model. This was done by estimating maximum likelihood (ML) of the model using MLwiN (version 2.00). The change in deviance is asymptotically distributed as χ^2 with corresponding change in degrees of freedom (Snijders and Bosker 1999). As we were mainly interested in within clutch effects, mean clutch values of the tested variables were included in the model yielding separate estimates for within and between clutch effects (Snijders and Bosker 1999). To increase statistical power, we controlled for laying date (1st egg of clutch), clutch size, laying order (as a categorical variable, because effects may not be linear), egg size and year (for the jackdaws) in the analyses, by adding each as a fixed variable to the model.

Results

We found no sexual difference in embryonic period in either jackdaws or gulls (Fig. 1). In both species the embryonic period decreased with laying order and laying date (Table 1). Although an effect of laying date of the first egg was only found in the data set from gulls hatched in the incubator, laying date was retained in the model, because of its significant interaction with laying order in all three data sets. This interaction was caused by an increase in hatching asynchrony later in the season. Embryonic period decreased with increasing clutch size in jackdaws (in gulls all clutches consisted of three eggs). In the jackdaws, embryonic period was slightly shorter in 2000 compared to 1998, and we controlled for this in our analysis. Interactions between sex and laying order were non-significant in all cases. There was no effect of egg size on embryonic period in jackdaws, either within clutches or between clutches. In

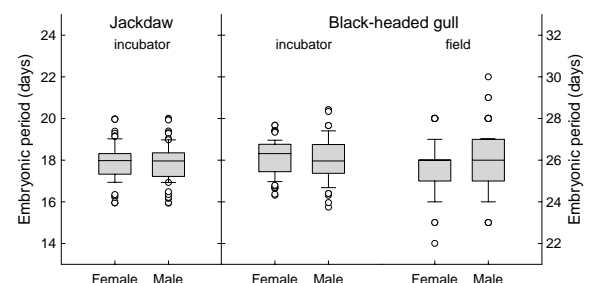


Fig. 1. No sexual difference in embryonic period was found in a) jackdaws b) black-headed gulls hatched in an incubator or c) black-headed gulls hatched under natural circumstances. Boxes show the median and the 25th and 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles and dots indicate outliers.

the gulls there was no effect of egg size on embryonic period among eggs hatched in the incubator, but embryonic period of clutches hatched in the field increased with increasing egg size, while within clutches relatively large eggs hatched earlier. There was no significant interaction between sex and egg size.

Discussion

We found no sexual difference in the duration of the embryonic period in jackdaws and black-headed gulls, which contrasts with the results obtained in kestrels (Blanco et al. 2003) and in black guillemots (Cook and Monaghan 2004), while two separate studies on domestic chickens reported contradicting results (Burke 1992, Dunnington et al. 1993). It is unlikely that lack of statistical power explains the absence of an effect, since our sample sizes were substantially larger than in previous studies and measurement precision of embryonic period was at least comparable. Part of the eggs used in this study hatched in an incubator, but this is unlikely to have affected the results. Eggs were in the incubator for only a small part of the embryonic period, and for gulls there was no difference between eggs hatching under natural circumstances or in an incubator. Hatching asynchrony increased in later clutches, causing a significant interaction between laying date and laying order. This was most likely caused either by an earlier start of incubation in the course of the season, a decrease in incubation after the hatching of the first chick (in the field), or both.

In contrast to previous studies we compared embryonic period of the sexes within clutches, which yields higher statistical power, because it controls for potential environmental and genetic effects on embryonic period. We also believe that, at least in the context of sex allocation, within-clutch comparisons are more relevant than between-clutch comparisons, because only within-clutch effects of sex on embryonic period affect sex allocation. In conclusion, our results indicate that a sexual difference in embryonic period is not a general pattern in birds, and therefore more studies are needed to establish how general sexual differences in embryonic period are.

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